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Review

Mechanism of PANoptosis in metabolic dysfunction-associated steatotic liver disease

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ABSTRACT

In recent years, the incidence of metabolic dysfunction-associated steatotic liver disease (MASLD) has been steadily rising, emerging as a major chronic liver disease of global concern. The course of MASLD is varied, spanning from MASLD to metabolic dysfunction associated steatohepatitis (MASH). MASH is an important contributor to cirrhosis, which may subsequently lead to hepatocellular carcinoma. It has been found that PANoptosis, an emerging inflammatory programmed cell death (PCD), is involved in the pathogenesis of MASLD and facilitates the development of NASH, eventually resulting in inflammatory fibrosis and hepatocyte death. This paper reviews the latest research progress on PANoptosis and MASLD to understand the mechanism of MASLD and provide new directions for future treatment and drug development.

1. Introduction

Recently, related theories have proposed MAFLD. Its concept is developed from the original NAFLD. At present, what we formerly referred to as non-alcoholic fatty liver disease (NAFLD) has been replaced with the term metabolic dysfunction-associated steatohepatopathy (MASLD) [1].It is a chronic disease that combines liver fat accumulation and metabolic disorders [2]. A "positive" diagnosis is based on histological or radiographic evidence of fatty accumulation in the liver and co-existence with obesity, diabetes, or metabolic disorders [3].

As one of the most prevalent chronic liver diseases worldwide, MASLD affects 30 % of the global adult population. Between 1991 and 2019, the prevalence increased from 22 % to 37 %, posing a significant risk to public health, and the prevalence varies from region to region [2-5].The prevalence rate in the United States is 32 %, and the overall prevalence rate in Asia is 29.62 % [6,7]. Among them, the prevalence of MASLD in Chinese adults is 29.81 %, more than a quarter [7]. The lowest incidence rates were in Africa at 13.48 % [8,9]. Regional differences in prevalence may be related to an ethnic group, socioeconomic, caloric intake, and so on [10,11].

At present, MASLD is widely recognized that it is the liver manifestation of metabolic syndrome and is related to metabolic factors such as hypertension, diabetes, and dyslipidemia. Although MASLD in its early stages typically doesn't inflict significant harm on the human body, it is a commonly held view that 20 % of people develop from MASLD to metabolic dysfunction-associated steatohepatitis (MASH), a more severe form of MASLD characterized histologically by the presence of lobular inflammation and hepatocellular ballooning [12]. And about 20 % of MASH is linked to an increased risk of cirrhosis [6]. The mortality rate of MASH-related cirrhosis is very high, about 30 % to 40 %, and 1.5 % to 2 % of people develop liver cancer in cirrhosis each year [6].Therefore, understanding the underlying mechanisms of this progression is crucial to elucidating the pathophysiology of MASLD and devising effective therapeutic strategies to mitigate its adverse consequences.

2. Physiopathological mechanisms of MASLD

Its pathophysiological mechanism is relatively complicated. About two decades ago, the "two-hit" theory was first proposed to explain the genealogical pathogenesis of MASLD. The theory suggests that MASH requires a double whammy, the "first hit" being steatosis of the liver, which reduces insulin sensitivity and turns into insulin resistance [13-15]. Then the liver cells are more susceptible to the "second hit". The "second hit" is damaging factors, such as oxidative stress (OS) and mitochondrial dysfunction, expression of pro-inflammatory factors and adhesion factors, apoptosis etc. These damaging factors can across the defense mechanism of cells and trigger inflammation, cell death, and

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fibrosis, promoting fatty liver to develop into MASH even cirrhosis and liver cancer [13,14,16].

As times progressed, the theory of "multiple strikes" was put forward, in which hepatocytes are susceptible to superimposed damage from a variety of factors, and there are many different parallel processes that contribute to MASH [17,18]. These different parallel processes include intestinal flora, toxic effects of adipose tissue, endoplasmic reticulum stress, etc. [16-19]. This theory points out that inflammation may precede steatosis, and MASH that progresses from steatosis may be the result of failure to resist lipotoxicity [17].

Both theories view MASLD as a disease that develops from MASH and insulin resistance, and that liver damage progresses linearly over time from less severe to more severe forms [20]. However, the "distinct hit" theory has recently been proposed, which suggests that this progression may be non-linear. In other words, the development of MASLD Spectrum is not simply the accumulation of lipid toxic damage. On the spectrum of MASLD, each level is a single disease that develops along a different pathway [20]. It regards MASLD and MASH as two independent diseases, not a real continuum of histological changes of the liver parenchyma. The genetic entity of MASH is different from other disease spectrum [20]. Its development is inextricably linked to apoptosis and necrosis during hepatocyte death. These extensive body of theories above illustrates the complexity of the disease and the multifactorial factors that drive its development, offering a deeper understanding of the etiology of MASLD [18].

3. Introduction to PANoptosis

3.1. Definition and characteristics of PANoptosis

PANoptosis is a novel mode of programmed death that was proposed by American scholars Malireddi et al. in 2019 and has gained significant attention as a new research hotspot in recent years. It is closely related to immune regulation, homeostasis maintenance, and embryonic development. A series of upstream molecular and receptor signals control PANoptosis. These signals combine to form polymeric complexes called PANoptosomes [21]. It possesses the essential characteristics of all three processes—pyroptosis, apoptosis, and necroptosis—which are where the letters "P," "A," and "N" in PANoptosis originate, but none of them can explain it by itself [22,23]. Current research on PANoptosis is mainly based on the pathogen infection model, but a growing number of studies have shown that it is also involved in the development of many other diseases [23]: such as Crohn's disease [24], and neurodegenerative diseases such as Parkinson's, Alzheimer's, and Huntington's diseases [25]. The relationship between pyroptosis and tumours has also been a major research interest [26]. Take caspases as an example. While CASP3 is highly expressed in malignant breast cancer, CASP1 has a tumor-suppressive effect in prostate, renal, and colorectal cancers. The caspase-8 gene is typically silent in malignant neuroendocrine tumors like neuroblastoma, medulloblastoma, and small cell lung cancer [27]. Numerous studies have shown that apoptosis, pyroptosis, and necroptosis also play an essential role in the pathogenesis of the spectrum of liver injuries in the progression of MASLD to MASH [24,28-32]. Consequently, it's critical to have a thorough comprehension of PANoptosis mechanisms for comprehending the onset and advancement of diseases, as well as for crafting novel therapeutic strategies.

3.2. PANoptosis in programmed cell death

Regulatory cell death exhibits distinct biochemical, morphological, and immunological properties. It is mediated by a signaling cascade involving effector molecules. Programmed cell death (PCD), a fundamental biological process regulating cell survival, tissue homeostasis, and various physiological and pathological conditions, is synonymous with regulatory cell death occurring under physiological conditions. PCD serves as the initial defense mechanism of the innate immune system. PCD, as one of the mechanisms through which the body preserves the integrity of the internal environment, initiates a suicidal response to stimuli induced by metabolic abnormalities. Excessive reactions disrupt the delicate balance between cell division and death, resulting in liver damage and disturbance of its inherent internal environment. This process also initiates a cascade of events, including stellate cell activation and immune cell recruitment [33], potentially contributing to the development of MASLD. Apoptosis, necroptosis, pyroptosis, PANoptosis, autophagy, ferroptosis, cuproptosis, and various other processes constitute the predominant types of PCD. Different physiological effects, ranging from morphological to biochemical changes, are linked to each type, induced by specific molecules [31,34].

Once thought to be associated with Parkinson's disease, apoptosis is a strictly regulated process essential for preserving tissue homeostasis and eliminating damaged or undesired cells. Cell shrinkage, chromatin condensation, DNA fragmentation, and the formation of apoptotic bodies—subsequently phagocytosed by nearby cells or macrophages without eliciting inflammation—are the hallmarks of apoptosis. It is initiated by signals originating from within the cell or from external sources that activate specific receptors on the outer cell membrane [35]. It is essential for various physiological functions, including tissue remodeling, regulation of the immune system, and embryonic development. Further recent research has shown that apoptosis contributes to the pathophysiology of MASH hepatocytes and is implicated in MASLD [36] .

Molecular signaling pathways are shared between necroptosis and apoptosis. However, necroptosis provides an alternative mechanism for programmed cell death because it can be activated by a combination of death ligands and death receptors when caspases are inhibited. It depends on the formation of the necrosome complex, comprising the Mixed lineage kinase domain-like protein (MLKL) and receptor-interacting protein kinases (RIPKs) [37]. Necrotic apoptosis and necrosis share similar morphological characteristics, including early membrane integrity rupture, cellular enlargement, and the subsequent release of intracellular organelles, possibly accompanied by additional inflammatory reactions [38]. Furthermore, there is increasing evidence that all necrotic apoptotic mediators have additional roles beyond necrosis that influence immunological responses, host defense against infections, and inflammatory diseases, while also contributing to metabolic regulation. This complexity adds to understanding necroptosis in the pathophysiology of metabolic diseases [36].

Pro-inflammatory cytokine production and inflammatory caspase activation are hallmarks of pyroptosis.t is an antimicrobial defense mechanism that functions similarly to apoptotic DNA breakage and is typically induced by microbial infection or cellular stress. On the other hand, pyroptosis causes cell enlargement, membrane rupture, and the release of inflammatory intracellular materials, all of which aid in the recruitment and activation of immune cells [39].As a result, pyroptosis is essential for host defense, inflammation, and the development of many inflammatory and infectious disorders.

Currently, the prevailing research perspective maintains that distinct forms of PCD are treated variably, regarded as unique entities operating in distinct settings, and investigated for possible therapeutic targets along different pathways. However, it is also possible for multiple forms of PCD cell death patterns to coexist. PANoptosis includes apoptosis, necroptosis, and pyroptosis. As the field of study develops, an increasing amount of evidence points to an overlap and crosstalk between these three modes of cell death, and they can cross-regulate each other. For instance, the apoptosis executors Caspase-3 and Caspase-7 can cleave GSDME, altering the morphology of cell death from apoptosis to pyroptosis. They can also inactivate GSDMD through amino-terminal splitting [40]. The execution of necrotic apoptosis and the formation of MLKL pore can activate apoptosis through membrane damage [41, 42]. These discoveries led to the establishment of the notion of PANoptosis, and the creation of focused treatment plans for MASLD requires

an understanding of the mechanism and management of PANoptosis.

4. PANoptosis in MASLD

MASLD initially presents as hepatic lipid accumulation without significant inflammation or fibrosis. The liver undergoes a spectrum of pathological changes influenced by multiple factors, ranging from simple steatosis to MASH. These changes signify the initiation of a critical stage characterized by hepatocellular injury, inflammation, and fibrosis, during which hepatocytes often succumb due to inflammatory processes [6]. When in an inflammatory state, the M1/M2 ratio of macrophages in adipose tissue changes to a certain extent [43]. Hypertrophic and inflammatory adipose tissue is the primary source of pro-inflammatory cytokines, such as TNF-α and IL-6, which can cause inflammatory responses through blood circulation to the liver [44].

PANoptosis plays a role in lipid metabolism, redox regulation, and other essential cellular functions. Exploring the functioning of pan-apoptosis in MASLD-MASH involves understanding how apoptosis, pyroptosis, and necroptosis intersect with hepatocyte death, highlighting the potential for discovering novel targets for therapy through PANoptosis. The specific molecular mechanisms are outlined below (Fig. 1).

First of all, we make a detailed discussion on the related mechanism of apoptosis. Many factors that trigger apoptosis, including endoplasmic reticulum, microsomes, peroxisome, and exogenous pro-apoptotic factors, but almost all of them work around mitochondria. When the mitochondria are affected by various harmful elements, the mPTP on the mitochondrial membrane is damaged. The mPTP permeability is reduced, resulting in the leakage of Cyt c [45]. Cyt c enters the cell-matrix and combines with Apaf-1 and pro-Caspase 9 to form the

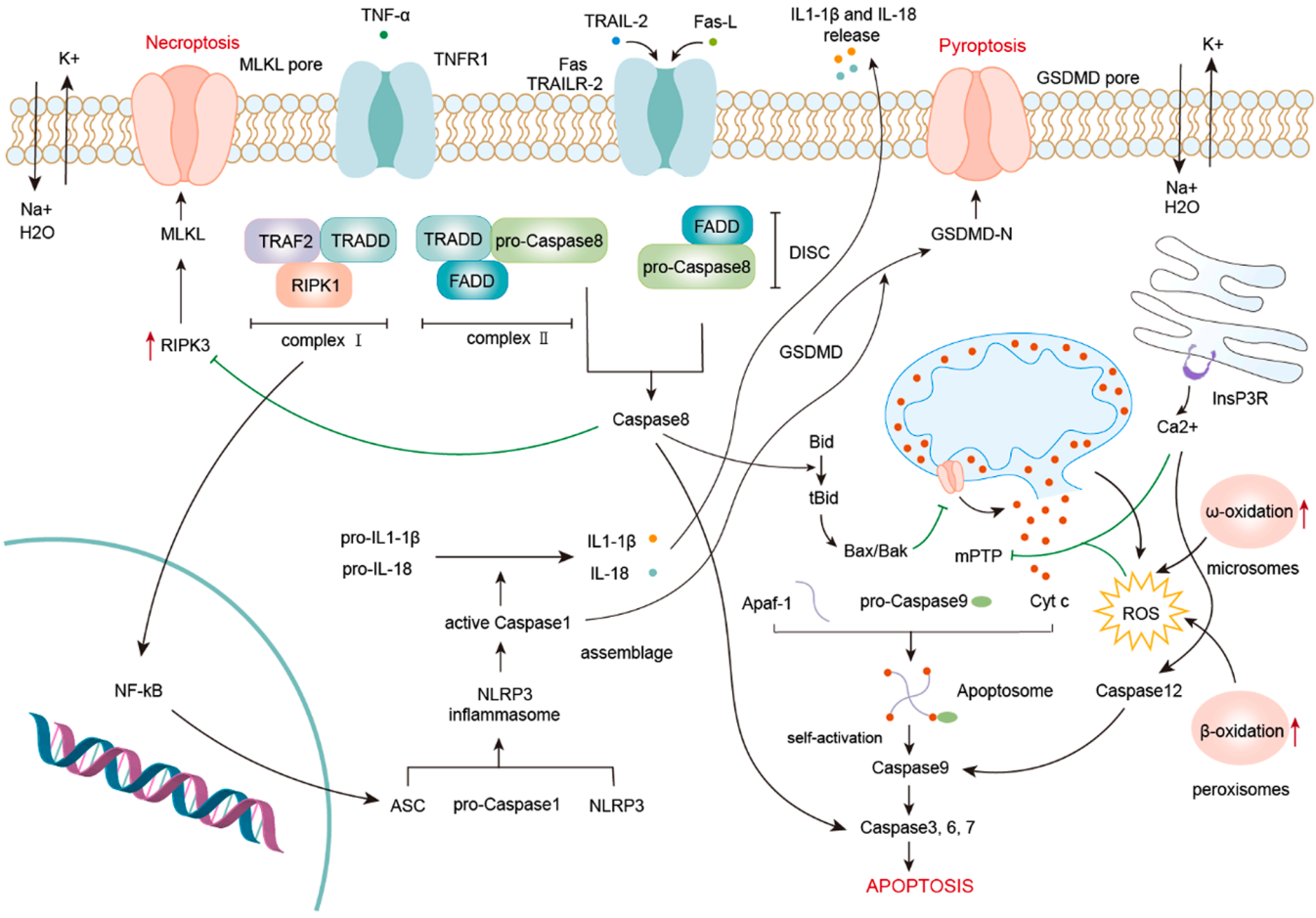

Fig. 1. Hepatocellular PANoptosis in MASH. There are three types of hepatocyte death in MASH patients.1. Mitochondrial apoptosis pathway. Under the action of harmful factors, the mPTP on the mitochondrial membrane is damaged, and the permeability is reduced, leading to Cyt c into the cell matrix. Cyt c combines with Apaf-1 and Pro-Caspase 9 to form the apoptotic body. The apoptotic body can self-activate to produce mature Caspase9, which activates downstream Caspase3, 6, 7 to trigger apoptosis. The permeability of mPTP is affected by several factors. (1) Excess ROS. Due to increased ω-oxidation activity of microsomes and increased β-oxidation activity of peroxisomes, excessive ROS are produced. (2) Excess Ca^{2+} is released. ERS results in excessive release of Ca^{2+} through $InsP_3R$ in ER reticulum. (3) Bax/Bak induced an increase in the permeability of mPTP. Exogenous factors trigger apoptosis by Bax/Bak. The in-cell matured Caspase8 can cleave Bid to produce tBid, and then activate Bax/Bak to produce the apoptotic effect of the mechanism mentioned above. In addition, Caspase 8 can directly activate Caspase 3, 6, 7 to cause apoptosis. Caspase8 can be activated by extracellular apoptotic factors in two ways.1) TNF-α acts on TNFR1, and downstream TRADD, FADD, and Pro-Caspase8 can be recruited to form complex II, followed by producing mature Caspase8. 2) TRAIL-2 and FAS-L act on the corresponding receptors TRAILR-2 and FAS on the liver membrane, and then downstream FADD and Pro-Caspase8 are recruited to form the DISC complex, followed by activated Caspase8.2. Pyroptosis. TNF-α acts on TNFR1, the downstream TRADD, TRAF2, and RIPK1 are recruited to form complex I, and NF-κB is subsequently activated to promote the expression of ASC. NLRP3 inflammasome, which consists of ASC, Pro-Caspase1, and NLRP3, can activate Caspase1. Caspase1 can make convert pro-IL-1β and pro-IL-18 to mature IL-1β and IL-18, respectively. Activated Caspase1 cleaves GSDMD to produce GSDMD-N fragments, and GSDMD-N forms GSDMD pores on the cell membrane. IL-β and IL-18 are released through the GSDMD pore to increase inflammatory substances in the liver. In addition, the formation of GSDMD pore leads to outflow of K^+, the influx of Na^+ and H_2O, swelling and rupture of hepatocytes, and subsequent death.3. Necroptosis. TNF-α acts on TNFR1, and downstream TRADD, TRAF2, and RIPK1 are recruited to form complex I to activate RIPK3. And then RIPK3 activates MLKL to form MLKL pore on the cell membrane and induce necrotic apoptosis. Necroptosis is similar to pyroptosis in causing hepatocytes death.

apoptotic body [46-48]. The apoptotic body can self-activate to produce mature Caspase9, which activates downstream Caspase3, 6, and 7 to trigger apoptosis [47,49].

Various factors, such as ROS accumulation and Ca^{2+} concentration, can affect the permeability of mPTP in mitochondrial membrane. Due to the excessive accumulation of fat in hepatocytes of NASH patients, the ω-oxidation activity of microsomes and the β-oxidation activity of peroxisomes increase and produce excessive ROS [50]. Of course, this also includes ROS produced by mitochondrial β-oxidation. In addition, ERS usually occurs in NASH, resulting in excessive release of Ca^{2+} into the cytoplasm through $InsP_3R$ in ER reticulum [51-53]. Studies have shown that excessive Ca^{2+} and ROS can lead to long-term opening and depolarization of mPTP [50,54,55]. And long-term opening and depolarization of mPTP can release apoptotic factors including $Ca^{2+}$and Cyt c into the cytoplasm [54,55]. In addition, the increase of mPTP opening can lead to imbalance of osmosis, leading to swelling of the mitochondrial matrix and rupture of the outer mitochondrial membrane [55]. A large amount of Cyt c is released from the break of the outer mitochondrial membrane, triggering the downstream apoptosis process [55].

Another cause of the excessive release of Cyt c from mitochondria is Bax/Bak [56,57]. Bax can be assembled into large oligomers that form porosity in the mitochondrial outer membrane [49]. And related research shows that Bax and Bak may work together to trigger the opening of mPTP. If we trace back to the root, we find that exogenous factors trigger apoptosis through Bax/Bak [48]. TNF-α can act on TNFR1, and downstream TRADD, FADD, and Pro-Caspase8 can be recruited to form complex II, producing mature Caspase8 [46]. TRAIL-2 and Fas-L also work on the corresponding receptors TRAILR-2 and FAS on the liver membrane, and then downstream FADD and Pro-Caspase8 are recruited to form the DISC complex, followed by activated Caspase8 [58,59]. The activation of Caspase8 in both pathways is derived from extracellular pro-apoptotic factors. The in-cell matured Caspase8 can cleave Bid to produce tBid, and then activate Bax/Bak to produce the apoptotic effect of the mechanism mentioned above [60]. And some studies have found that Caspase 8 can directly activate Caspase 3, 6, 7 to cause apoptosis [47,49].

It is widely recognized that apoptosis leads to hepatocyte death in NASH, but pyroptosis also plays a vital role in hepatocyte death. After TNF-α acts on TNFR1, the downstream TRADD, TRAF2 and RIPK1 are recruited to form complex I, and NF-κB is subsequently activated to promote the expression of ASC [33,61]. ASC, Pro-Caspase1, and NLRP3 assemble to form NLRP3 inflammasome, leading to caspase1 activation [62,63] Caspase1 can make convert pro-IL-1β and pro-IL-18 to mature IL-1β and IL-18, respectively [64]. Activated Caspase1 cleaves GSDMD to produce GSDMD-N fragments, and GSDMD-N forms GSDMD pores on the cell membrane [58,65,66]. IL-1β and IL-18 are released through GSDMD pores to increases the inflammatory substances in the liver [61, 67]. What's more, the formation of GSDMD pore leads to outflow of K^+, and inflow of Na^+ and H_2O, resulting in swelling and rupture of hepatocytes, followed by death [61].

The final mechanism leading to hepatocyte death is necroptosis, distinguished from necrosis by programmed death. Pathological hepatocyte death in NASH can produce damage-associated molecular patterns DAMPs[2]. DAMPs, including ATP, uric acid, and cholesterol, not only play an inflammatory priming effect by activating Kupffer cells but also in turn, activate hepatocytes and play an inflammatory cascade effect [15,68,69]. Kupffer cells are a type of macrophages that play a crucial role in liver inflammation [70]. It is known that there are two modes of DAMPs and PAMPs, both of which can activate Kupffer cells in the liver, causing NASH or further inflammation [71,72]. The expression of NF-κB and inflammasome NLRP3 in Kupffer cells increased, leading to the expression and release of downstream inflammatory factors TNF-α, IL-6, and IL-1β [72-77]. TNF-α acts on TNFR1, and downstream TRADD, TRAF2, and RIPK1 are recruited to form complex I to activate RIPK3, and then start MLKL to create MLKL pores on the cell membrane and induce necrotic apoptosis [36,67,78-82]. Similar to how pyrophosis causes death, its MLKL hole triggers the outflow of K^+ and the inflow of Na^+ and H_2O [81]. The liver cells swell and rupture, and then die [79].

5. Therapeutic implications and future directions

Over the past decade, the medical community has enhanced its understanding of MASLD, transitioning from NAFLD to MASLD and from exclusionary to definitive diagnosis. Nevertheless, research on MASLD treatment options continues. Primary treatment strategies for MASLD typically commence with lifestyle interventions (e.g., diet, physical activity, weight management, and smoking cessation), potentially resolving SLD in up to 97 % of patients [83]. Furthermore, appropriate assessment and management of metabolism-related conditions such as cardiovascular disease and diabetes can also mitigate fatty liver. Pioglitazone and GLP1 RAs are the two anti-hyperglycemic drugs with demonstrated efficacy and safety in reversing MASH [84,85].However, as of now, there are no FDA-approved drugs for treating MASLD and MASH [86]. Drug development in MASLD trials poses challenges due to the considerable variability in histopathological assessment of liver biopsies [87]. The introduction of the concept of PANoptosis has prompted a transition from apoptosis to non-apoptotic modes of cell death, such as pyroptosis and necroptosis, which formerly played a significant role in MASLD. The pro-inflammatory response triggered by PANoptosis-mediated modulation of immune cells may indeed be the primary driver of liver inflammation. Consequently, targeted intervention in these modes of cell death has emerged as a focal point in MASLD research and is also viewed as a promising therapeutic avenue.

Recently, it has been suggested that liproxstatin-1, a ferritin deposition inhibitor, attenuates steatosis and steatohepatitis by blocking PANoptosis [88], thus offering new insights into targeted therapy for MASLD. Ezquerro et al. also demonstrated that an increase in acylated/deacylated ghrelin ratio may potentially benefit the progression of MASLD by counteracting the compensatory mechanism of TNF-α-induced apoptosis, necrosis, and pyroptosis in hepatocytes [89], thereby attenuating inflammation and hepatic injury induced by PANoptosis. NLRP3 plays a pivotal role in the PANoptosis progression from benign MASLD to inflammatory MASH. Several studies in various preclinical diet-induced MASH mice models have demonstrated that the administration of an NLRP 3 inhibitor (MCC 950 or IFM-514) alleviated the severity of hepatic inflammation and fibrosis [62,90]. MCC 950 (also known as CP-456 or CRID 3) is a potent and selective small-molecule inhibitor of NLRP 3, capable of inhibiting ATP hydrolysis for NLRP 3 inflammasome function, thereby blocking NLRP 3 [91,92]. This implies a potential role for NLRP 3 inhibition in NASLD treatment [93]. However, despite the demonstrated efficacy of MCC 950 and IFM-514 in preclinical models, clinical trials for MASH treatment have not yet commenced. Additionally, various potential therapeutic strategies based on PANoptosis regulatory pathways and related signaling pathways, including antioxidants targeting the mitochondrial pathway, anti-inflammatory agents targeting the death receptor pathway, and compounds targeting the endoplasmic reticulum stress pathway, have demonstrated therapeutic potential. Future research should prioritize a comprehensive understanding of PANoptosis regulatory pathways, and the development of clinical studies and therapeutic interventions directed at these pathways.

6. Conclusion

The prevalence of NAFLD has increased at an alarming rate and has become the most common liver disease today. NASH is closely associated with obesity and metabolic syndrome and is a leading cause of cirrhosis and liver cancer. Its pathogenesis and mechanism are very complex, involving environmental and genetic effects. PANoptosis, a novel mode of PCD, has garnered increasing attention from a wide range of researchers. The association between PANoptosis and metabolic disorders has been evidenced in a growing body of literature, suggesting

that PANoptosis holds promise for innovative treatment strategies for these disorders. Further investigation in diverse animal models and clinical cohorts is justified given the presence of PANoptosis in both MASLD and MASH. It is anticipated that these investigations will elucidate the regulatory mechanisms underlying PANoptosis, thereby providing a robust foundation for addressing MASLD and advancing novel drug development.

CRediT authorship contribution statement

Keying Ni: Writing – review & editing, Writing – original draft. **Lina Meng:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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